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EFFECT OF N-TRIACONTANOL ON THE GROWTH OF SALT STRESSED SOYBEAN PLANTS

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Abstract: The present investigation was made to describe the effect of foliar application of n-triacontanol on plant growth, physiological and biochemical parameters in soybean under sodium chloride (NaCl) stress. The seeds were propagated into the soil supplemented with 10, 15, 20, 25 and 30 mM NaCl. A moderate reduction of plant growth was observed in 20 mM NaCl treatment compared to other concentration of NaCl treatments. Hence, the n-triacontanol was sprayed on the plants grown under the 20 mM NaCl added soil. Increased in specific leaf area (SLA), leaf weight ratio, relative water content and decreased in leaf osmotic potential were noted in n-triacontanol treated plants than that of salt stressed untreated plants. Moreover, the chlorophyll pigments, nucleic acids, total soluble sugars and proteins were also found to be increased in the n-triacontanol treated plants. The accumulation of proline was decreased in salt stressed plants that had been treated with n-triacontanol. The present study effectively proved that n-triacontanol was able to restore the normal metabolic process in the salt stressed soybean.

Keywords: NaCl Stress, N-triacontanol, Soybean

INTRODUCTION

High soil salinity or drought is the major environmental factor, which reduce the crop yield in the cultivated lands. The excess amount of soluble inorganic salts could alter the nature of soil through soil aeration, water and texture, and also affect the living organisms. Nearly 20% of the world's cultivated area is affected by soil salinity. Salinity leads to various metabolic disturbances resulting in general suppression of seed germination, plant growth and yield (Chandrasekar & Sandhayarani 1996; Sharma & Saran 1994). High concentration of ions in the external solution (Na^+, Cl^- or Ca^{2+}) that are taken up by roots at high rates may lead to excessive accumulation in the tissue. These ions may inhibit the uptake of other ions into the root (such as K^+) and their transport to the shoot through the xylem, eventually leading to a deficiency in the tissue. Thus, there is the potential for many nutrient interactions in salt stressed plants which may lead to important consequences for growth (Gadallah 1996). Application of abiotic stresses resulted in an altered level of plant growth hormones that decreased plant growth (Gupta *et al.* 1993). Soybean is an important economical and medicinal plant. However, the yield of this plant is reduced due to high salinity in the soil. Therefore, reduction in plant growth under stress conditions could be an outcome of altered hormonal balance and hence, their exogenous application

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provides an alternative approach to counter the stress conditions (Kaur *et al.* 1998). Chen *et al.* (2002) reported that the exogenous application of n-triacontanol increased the plant growth and yield.

Triacontanol (TRIA), a saturated primary alcohol, is a natural component of plant epicuticular waxes (Chibnall *et al.* 1933; Crosby & Vlitos 1959) and has plant growth enhancing properties (Ries *et al.* 1977). TRIA is used to increase crop yields on millions of hectares, particularly in Asia. Many researchers have reported the enhanced growth and yield of plants with application of TRIA (Nagoshi & Kawashima 1996; Muthuchelian *et al.* 1997; Borowski *et al.* 2000; Kumaravelu *et al.* 2000; Chen *et al.* 2002). When applied in field conditions, TRIA also showed an increase in vegetative growth, chlorophyll content and dry weight of various plants (Ries 1985). To date there is no information pertaining to the effects of foliar applications of TRIA on growth characters and biochemical changes in soybean plants under salt stressed conditions. Hence, our objective was to study the effect of n-triacontanol on the growth of salt stressed soybean plants.

MATERIALS AND METHODS

Plant Material and Treatments

The certified seeds of Soybean CO 1 variety were obtained from Tamilnadu Agricultural University, Coimbatore, India. These seeds were surface-sterilized with 0.1% $HgCl_2$ solution for 5 min and washed thoroughly five times with distilled water. The seeds were propagated in claypots containing air-dried red soil (control) and supplemented with 10, 15, 20, 25 and 30 mM of NaCl. The pots were maintained under natural photoperiod and then watered regularly. After 10 days, fresh weight, dry weight, and length of roots and shoots were measured.

Based on the preliminary experiment results, 20 mM NaCl was selected as the saline stress concentration for subsequent experiment. After 10 days of seed germination on 20 mM of NaCl supplemented soil, 10 mM n-triacontanol (50 ml per pot) was sprayed once in every two days interval for a period of 20 days.

Analysis of Physiological Parameters

After 25 days from seed germination, the leaves were collected and SLA was calculated using formula of leaf area/leaf dry weight; leaf weight ratio was measured by total leaf dry weight/total plant dry weight; the plant water status was analyzed by leaf osmotic potential according to the method of Kramer (1969) and the relative water content as reported by Turner (1981).

Analysis of Biochemical Parameters

Photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophylls were extracted and estimated adopting the methods described by Arnon (1949). Total soluble sugars in leaves were determined by the method of Dubois *et al.* (1956).

The leaves were homogenized using 70% (v/v) ethyl alcohol and were utilized for estimation of soluble proteins and nucleic acids. The homogenate was precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was then dissolved in 1% (w/v) sodium hydroxide (NaOH) solution. Quantitative estimation of protein was done employing the method of Lowry *et al.* (1951). DNA and RNA were isolated from the ethyl alcohol homogenate by the method of Ogur and Rosen (1950) and were quantified according to the methods of Burton (1968) and Schneider (1957) respectively. The leaves were also homogenized using 30% sulphosalicylic acid to estimate the proline according to Bates *et al.* (1973).

Statistical Analysis

Data from experiment, fresh and dry weight of the plant, length of shoot and roots, SLA, leaf weight ratio, relative water content, leaf osmotic potential, chlorophyll pigments, total soluble sugar and protein, nucleic acids and proline content of the control, salt stressed and n-triacontanol treated salt stressed plants were analyzed for the standard error and significance of variance by one-way ANOVA. A statistical analysis was carried out using SPSS software and the level at 0.05 was considered as significant.

RESULTS

Effect of Salt Stress on Growth of Seedlings

The fresh and dry weight, root and shoot length were studied under control and saline conditions (10, 15, 20, 25 and 30 mM NaCl). The gradual decreased in fresh and dry weight, root and shoot length of seedlings with increasing concentrations of NaCl treatments were shown in Figures 1 and 2. When the seedlings grown under high salinity stress (30 mM NaCl), the fresh (40%) and dry weight (32%), root (23%) and shoot length (28%) were highly reduced when compared to the control plants. However only moderate decreased in fresh (26%) and dry weight (20%), length of roots (17%) and shoots (13%) were noticed in 20 mM NaCl treated plant. Moreover, a lesser decreased of 9% and 18% were noticed in the fresh and dry weight, 15% and 17% decreased respectively, in root and shoot length at 10 mM NaCl treated seedlings compared to the control plants.

Effect of N-triacontanol and Changes in Physiological Characters

Application of n-triacontanol considerably restored the SLA, leaf weight ratio, leaf osmotic potential and relative water contents in plants grown in saline medium (Table 1). Compared to the control set, SLA of plants was reduced about 29.6% under 20 mM NaCl salt stress. The n-triacontanol was able to minimize the percentage reduction from 29.6% to 8.9%. The leaf weight ratio of salt stressed plants was less (63.5%) than the normal seedlings, while it was improved (63.5% to 25.4%) by the foliar application of n-triacontanol. Salt stress, which increased the osmotic potential (7.9%) of leaves of plants compared to the control plants; the n-triacontanol was found to decrease the leaf osmotic potential. N-triacontanol was found to be more effective in increasing the relative water

content (25.8%) in salt stressed plants compared to salt stressed plants without n-triacontanol treatment.

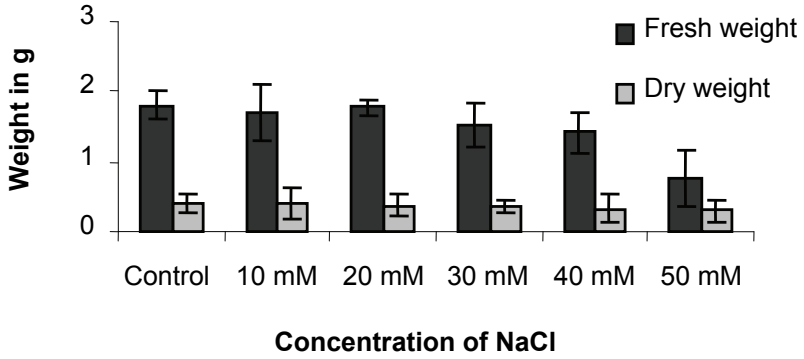

Figure1: Fresh and dry weight of control and NaCl stressed soybean plant.

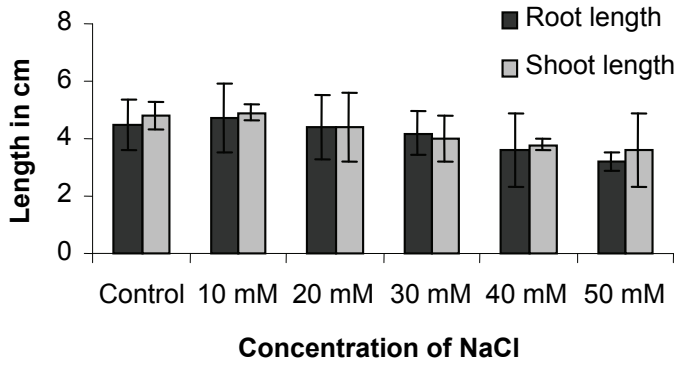

Figure 2: Root and shoot length of control and NaCl stressed soybean plant.

Table 1: The various physiological parameters in control, NaCl stressed and NaCl stressed with n-triacontanol treated soybean plants.

Treatment	SLA (cm^2g^{-1})	Leaf weight ratio (gg^{-1})	Leaf osmotic potential (bar)	Relative water content (%)
Control	688.0 ± 1.9	0.20 ± 0.7	3.0 ± 0.6	91.8 ± 1.4
NaCl	484.1 ± 3.1	0.07 ± 0.3	3.2 ± 0.7	73.3 ± 0.7
NaCl + n-triacontanol	626.7 ± 1.6	0.15 ± 0.2	3.1 ± 0.5	86.1 ± 0.9

Effect of N-triacontanol in Plant Biochemical Characters

The application of n-triacontanol considerably restored the pigment level in plants grown in saline soil (Fig. 3). The salt treated plants produced a decreased content of chlorophyll a (18.4%), b (23.8%) and total chlorophyll (24%) compared to the control plants. The level of chlorophyll a, b and total chlorophyll were found to increase in salinity affected plants treated with n-triacontanol.

An increase in total soluble sugar (1.6%) was observed in salt induced plant that had been treated with n-triacontanol when compared to salt induced untreated plant (Table 2). In NaCl stressed (without n-triacontanol untreated) plants, the soluble proteins decreased (20%) compared to control plants, while foliar spray of n-triacontanol increased (26.7%) the soluble proteins in plants under the salt stress. Nucleic acids (DNA and RNA) decreased in plants grown in saline soil than that of in normal soil. The application of n-triacontanol increased the contents of DNA (20%) and RNA (5.5%) in NaCl stressed plants. The proline content (49.5%) was increased in stress affected plants than control plants; while the proline content in the salt stressed plants subjected to n-triacontanol treatment was reduced (49.2% to 13.6%) (Table 2).

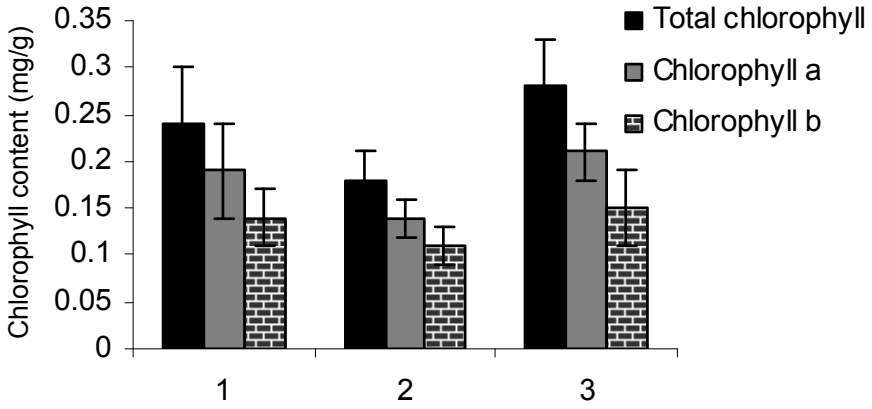

Figure 3: Photosynthetic pigments in control (1), NaCl stressed (2), and NaCl stress with n-triacontanol treated (3) soybean plant.

Table 2: The various biochemical parameters in control, NaCl stressed, and NaCl stressed with n-triacontanol treated soybean plants.

Treatment	Total soluble sugar (mg/g dry weight)	Soluble protein (mg/g dry weight)	DNA (mg/g dry weight)	RNA (mg/g dry weight)	Proline (mg/g dry weight)
Control	28.5 ± 2.7	20.2 ± 2.5	23.2 ± 1.8	66.2 ± 9.8	7.3 ± 0.3
NaCl	0.9 ± 1.1	16.2 ± 1.9	12.7 ± 1.9	38.2 ± 2.7	10.9 ± 0.9
NaCl + n-triacontanol	23.3 ± 1.7	20.4 ± 2.7	18.6 ± 1.4	62.5 ± 5.4	8.3 ± 1.0

DISCUSSION

The variations in the plant fresh and dry weight, roots and shoots length were measure in 10, 15, 20, 25 and 30 mM NaCl stressed soybean seedlings. In our experiment, 10 mM and 15 mM NaCl induced a mild stress, while 20 mM NaCl induced a moderate stress in plants grown in the saline conditions. Moreover, the plants were severely stressed in 25 mM NaCl and 30 mM NaCl added soil. Bhardwaj and Rao (1973) noticed that loss in dry weight in wheat and gram are varieties under salt stress. The presence of salt stress was found to reduce the biomass (fresh and dry weight) yields in Vigna cultivars (Sumithra *et al.* 2006). The present investigation observed the same phenomena that the plant fresh and dry biomass, and root and shoot length of seedlings were reduced in NaCl added soil.

The salt treated soil was found to reduce the SLA of the plants. The salt stress could adversely affect the cell division and enlargement of leaves resulting in reduced leaf area (Ravichandran & Mungse 1995). Foliar application of n-triacontanol increased the SLA and this could be due to acceleration of cell division and enlargement. As reported by Darra and Saxena (1971), the ameliorative role of growth regulators (GA_3) on leaf size enlargement might be attributed to accelerate cell division and enlargement.

The high uptake of Na^+ and Cl^- content decreased the leaf weight ratio of plants grown on salt stress. The presence of n-triacontanol could be enhance the nutrient uptake of salt stressed plants. The high uptake of essential nutrient such as K^+ and Ca^{2+} reduce the low uptake of Na^+ ions might also be responsible for the enhanced leaf growth (Banuelos & Bangerth 1986).

Osmotic adjustment is a major adaptive mechanism to stress. The leaf osmotic potential became increased in plants exposed to salt stress. The organic solutes increased in the leaves of plants grown in salt stress (Bal & Dutt 1986) might be the reason for increases in the leaf osmotic potential. Nandini and Subhendu (2002) reported that the osmotic potential could be increased due to excess of translocation of Na^+ and Cl^- in the leaf cells of salt stressed plants. The n-triacontanol treated salt stressed plants, osmotic potential was reduced than salt stressed plants. The n-triacontanol might be increase the K^+ and Ca^{2+} contents of the leaves. Whereas, Nandini and Subhendu (2002) observed the K^+ and Ca^{2+} contents of the hormone treated mung bean plants under salt stress were almost like control plants. Salt stress induced a reduction in the relative water content of the leaves, which indicates a loss of turgor that resulted in limited water availability for cell extension process (Katerji *et al.* 1997). The growth regulator n-triacontanol was able to overcome the stress effect.

In our experiment, a decreased in chlorophyll levels was observed in salt stressed plants. Similarly, the effect of salt stressed plants on reduction of chlorophyll contents had been reported in several plants, such as rice (Pandey & Saxena 1987) and tomato (Sinelnikova *et al.* 1998). Salinity decreased nitrogen availability which could be one of the reasons for decreased chlorophyll content (Parashar & Verma 1993). The reduction of total chlorophyll content was probably related to the enhanced activity of the enzyme chlorophyllase (Reddy & Vora 1986). The n-triacontanol was found to increase chlorophyll levels in salt

stressed plants. GA_3 and IAA were also found increased in the chlorophyll content of salinized wheat plant (Aldesuquy 1992). A promotion of growth and the increased level of chlorophyll content in salt stressed rice treated with brassinosteroids were observed by Anuradha and Seeta Ram Rao (2003).

Salt stress reduced the total soluble sugar as compared to the control plants. Qasim *et al.* (2003) observed the reduction in total soluble sugar in *Brassica nappa* L. grown under NaCl added soil. The total soluble sugar content increased in n-triacontanol applied salt stressed plants.

Salinity decreases the soluble protein and increases the protease activity (Murumkar & Chavan 1987). When the n-triacontanol was sprayed on the leaves, there was an increased in accumulation of soluble proteins in salinized plants. GA_3 has been reported to increase the soluble proteins in the leaves of a tree legume *Parkia javanica* (Premabati & Srivastava 1995).

The promotion of growth in rice seedlings by n-triacontanol under saline conditions was associated with enhanced levels of nucleic acids and soluble proteins. The NaCl treatment suppressed the synthesis of DNA and RNA contents and this effect was significantly alleviated by the foliar application of n-triacontanol. These results were supported by Anuradha and Seeta Ram Rao (2001) and they reported that the DNA and RNA contents decreased in rice seedlings due to the effect of salt stress, and the application of brassinosteroids to the stressed plants overcame the stress effect.

Role of proline in plant is related to survival rather than to maintenance of growth (Greenway & Munns 1980). In our experiment, the proline content was higher in salt stressed soybean. The foliar application of n-triacontanol suppressed the effect of salinity. Similarly, Singh *et al.* (1994) observed a declined in proline content with kinetin treatment in mung bean under saline conditions.

In conclusion, our study showed that SLA, leaf weight ratio, relative water content, chlorophyll pigments, nucleic acids, soluble sugars and soluble proteins were reduced in plants grown in saline substratum. The growth regulator n-triacontanol was able to reduce the effect of salt stress and increased plant growth and other biochemical parameters. Even though the leaf osmotic potential and proline accumulation were increased by the effect of salt stress, they decreased when the salt stressed plants were treated with foliar spray of n-triacontanol.

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